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BRIEF COMMUNICATION

The Role of ATP-Sensitive Potassium Channels in Striatal Dopamine Release: An In Vivo Microdialysis Study

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TANAKA, T., M. YOSHIDA, H. YOKOO, K. MIZOGUCHI AND M. TANAKA. *The role of ATP-sensitive potassium channels in striatal dopamine release: An in vivo microdialysis study.* PHARMACOL BIOCHEM BEHAV **52**(4) 831–835, 1995.—We used in vivo brain microdialysis to investigate the role of adenosine triphosphate (ATP)-sensitive potassium (K_{ATP}) channels in dopamine (DA) release regulated by DA autoreceptors in the rat striatum. Local infusions of the K_{ATP} channel opener nicorandil (10^{-5}–10^{-3} M) into the striatum through the dialysis membrane produced dose-dependent decreases in extracellular concentrations of DA. Local application of the D_2 receptor antagonist (−)-sulpiride (SLP, 10^{-5} M) or the K_{ATP} channel blocker quinine (QIN, 10^{-3} M) produced significant increases in extracellular concentrations of DA. Nicorandil (10^{-3} M) significantly blocked SLP (10^{-5} M)- or QIN (10^{-3} M)-induced increases in DA levels in the striatum. These results suggest that activation or inhibition of the K_{ATP} channel in the striatum causes decreases or increases, respectively, in endogenous DA release in vivo. Furthermore, SLP-induced increases in DA levels caused by blocking the tonic activation of DA autoreceptors are inhibited by the activation of K_{ATP} channels. These data suggest that striatal DA autoreceptors may inhibit DA release tonically by activating the K_{ATP} channel.

ATP-sensitive potassium channel Nicorandil Sulpiride Quinine Dopamine autoreceptors Microdialysis Striatum

ADENOSINE triphosphate (ATP)-sensitive potassium (K_{ATP}) channels are closed by the actions of intracellular ATP (17). These channels are found in pancreatic β cells (2) and cardiac muscle (17). Recent binding studies showed that K_{ATP} channels are also widely distributed in the CNS (8,16). Closing of K_{ATP} channels causes depolarization, which leads to insulin secretion from pancreatic β cells (24). On the other hand, opening of K_{ATP} channels relaxes smooth muscle by increasing potassium conductance, which results in hyperpolarization (9). However, little is known of the role of K_{ATP} channels in the CNS.

It has been shown that dopamine (DA) D_2 receptors in the substantia nigra (SN) are coupled to potassium channels. Activation of these channels by DA produces a reduction in the rate of firing in dopaminergic neurons (14,15). Recently, it has been suggested that the SN has the highest density of K_{ATP} channels in the brain (16,27) and that D_2 receptors in the SN produce hyperpolarization by activation of K_{ATP} channels (21). However, glibenclamide, which also blocks K_{ATP} channels (4), does not antagonize DA-induced hyperpolarization. Furthermore, the K_{ATP} channel opener, cromakalim, has no effect on dopaminergic neurons in the SN (11), which suggests that D_2 receptors may not be coupled to K_{ATP} channels. All these reports (4,11,14,15) employed in vitro models, and it is not clear from these reports (11,21) whether D_2 receptors are coupled to K_{ATP} channels in vivo. In vivo microdialysis is an

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effective technique for studying presynaptic DA autoreceptor function in vivo (12,26) and is a useful technique for applying small quantities of drugs to restricted brain regions (18).

The purpose of this study was to investigate the role of K_{ATP} channels in DA autoreceptor function in nigrostriatal dopaminergic nerve terminals, using the in vivo microdialysis technique.

METHODS

All animal procedures were performed in accordance with the Guiding Principles for The Care and Use of Animals in the Field of Physiological Sciences (Physiological Society of Japan) and were approved by the Committee of Animal Experimentation, Kurume University School of Medicine. Male Wistar rats (260–350 g) were anesthetized (sodium pentobarbital, 50 mg/kg, IP) and stereotaxically implanted with a U-shaped microdialysis probe in the right striatum. The coordinates for placement of the tip of the probes were: AP +0.5, ML 3.0, DV 6.5 mm for the striatum in relation to bregma and to the brain surface, according to the atlas of Paxinos and Watson (20). The active region of the cellulose hollow dialysis tube (0.25 mm diameter; mol. wt. cutoff of 50,000) was 5 mm in length. All experiments were carried out 1 or 2 days after the implantaion of the dialysis probe, on freely moving, conscious rats during the light phase of the day-night cycle.

The rat was connected directly to the high-performance liquid chromatographic (HPLC) equipment for on-line analysis of DA (Eicom Co, Kyoto, Japan). The dialysis tube was perfused with a solution (NaCl 140 mM, KCl 3.35 mM, $MgCl_2$ 1.15 mM, and $CaCl_2$ 1.26 mM, pH 7.4) at a flow rate of 2.5 μl/min using a microperfusion pump. The dialysis sample (50 μl) was injected every 20 min via an autoinjecter (Eicom AS-10). The mobile phase consisted of 0.1 M sodium acetate, 0.1 mM EDTA, 0.7 mM octanesulfonic acid, and 10% methanol at pH 4.0. DA was separated on an Eicompack MA-ODS column (4.6 mm OD, 3.6 mm ID × 150 mm) at 25°C. The graphite working electrode was set at +600 mV vs. an Ag/AgCl reference electrode (Eicom ECD-100 electrochemical detector), and the flow rate (Eicom EP-10 pump) was 0.9 ml/min. The detection limit of DA was about 0.5 pg/20 min. An integrator (Chromatocorder 12, SIC, Hachiozi, Japan) was used to record the signal. After a stable baseline was obtained, all drugs were added to the perfusion fluid. The average of three baseline samples immediately preceding drug perfusion was defined as 100%, and all subsequent measures were related to these values (percent changes). In Experiments 2 and 3, during perfusion with nicorandil (NIC) (1 mM) alone for 1 h, DA values again became stable. For this reason, the three samples of the NIC infusion period were regarded as an appropriate baseline and defined as 100%. Differences between the baseline dialysate concentrations and the drug-infused samples were analyzed by one-way analysis of variance (ANOVA) followed by the Newman-Keuls test. The effects of NIC on quinine (QIN)- or sulpiride (SLP)-induced DA release were analyzed by two-way ANOVA followed by the Newman-Keuls test.

Experiment 1: Effect of Local Application of a K_{ATP} Channel Opener, NIC, a K_{ATP} Channel Blocker, QIN, and a D_2 Receptor Antagonist, SLP, on Extracellular Concentrations of DA

After a stable baseline was obtained, the K_{ATP} channel opener NIC (31) was infused through the dialysis probe at concentrations of 10^{-5}–10^{-3} M, each for 1 h (Fig. 1). The

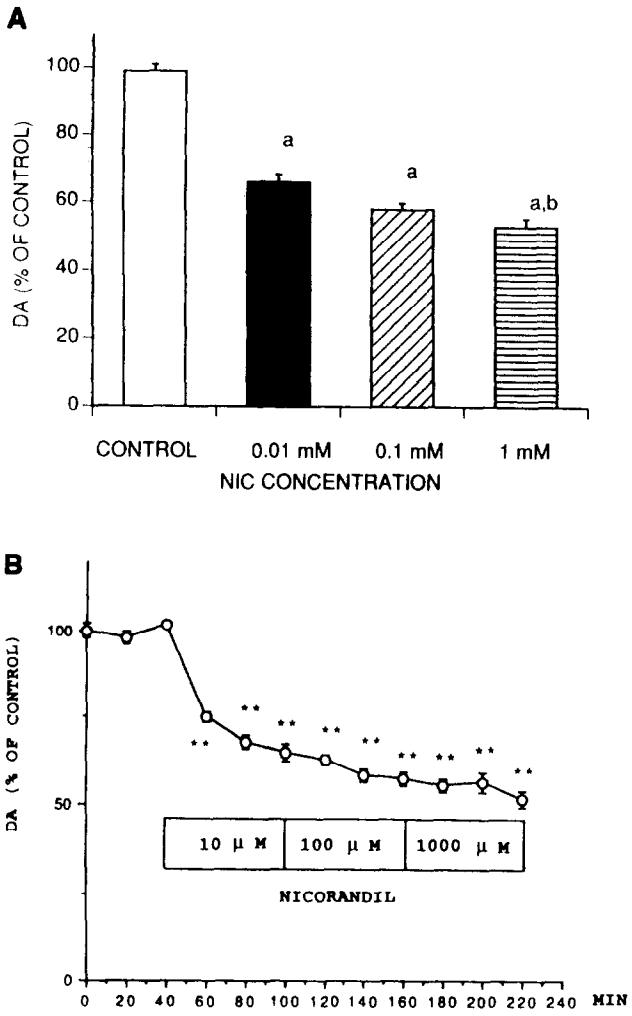

FIG. 1. Effect of various doses of nicorandil (NIC) on extracellular concentrations of DA in the striatum. NIC was perfused through the dialysis membrane. Only the maximum decrease of DA was given (A). NIC produced a dose-dependent decrease in extracellular concentrations of DA. Each point represents the mean ± SEM (n = 4). (a) $p < 0.01$ compared to control (one-way ANOVA and Newman-Keuls test). (b) $p < 0.01$ compared to 0.01 mM NIC (Student's t-test). $^{**}p < 0.01$ compared to control (one-way ANOVA and Newman-Keuls test).

K_{ATP} channel blocker QIN (10^{-3} M) (6), or the D_2 receptor antagonist SLP (10^{-5} M) was added to the perfusion solution for 1 h after a stable baseline was obtained (Fig. 2).

Experiment 2: Effect of Local Application of a K_{ATP} Channel Opener on K_{ATP} Channel Blocker-Induced Changes in DA

After perfusion with NIC (10^{-3} M) for 1 h, QIN (10^{-3} M) was coinfused with NIC for a further 1 h (Fig. 2A).

Experiment 3: Effect of Local Application of a K_{ATP} Channel Opener on D_2 Receptor Antagonist-Induced Changes in DA

After perfusion with NIC (10^{-3} M) for 1 h, SLP (10^{-5} M) was coinfused with NIC for a further 1 h (Fig. 2B).

RESULTS

The average basal extracellular DA level detected in all animals used in these experiments (n = 20) was 2.6 ± 0.2 fmol/min.

Effect of NIC, QIN, or SLP Infusion on Extracellular Levels of DA

Local application of NIC (10^{-5}–10^{-3} M) produced decreases in extracellular DA concentrations in a dose-dependent manner. The maximum decrease in DA was 52% of baseline at a dose of 10^{-3} M (Fig. 1). Local infusion of QIN (10^{-3} M) into the striatum produced a significant increase in DA levels. The maximum increase in DA was observed in the first sample obtained in the presence of QIN (310%), after which DA levels decreased gradually (Fig. 2A). Local infusion of SLP (10^{-5} M) into the striatum significantly increased DA content to a maximum of 205% of the basal value (Fig. 2B). However, the time course of changes in DA level induced in SLP differed from that induced by QIN [i.e., SLP increased DA levels gradually and the maximum increase of DA was found in the last SLP infusion sample (205%, Fig. 2B)].

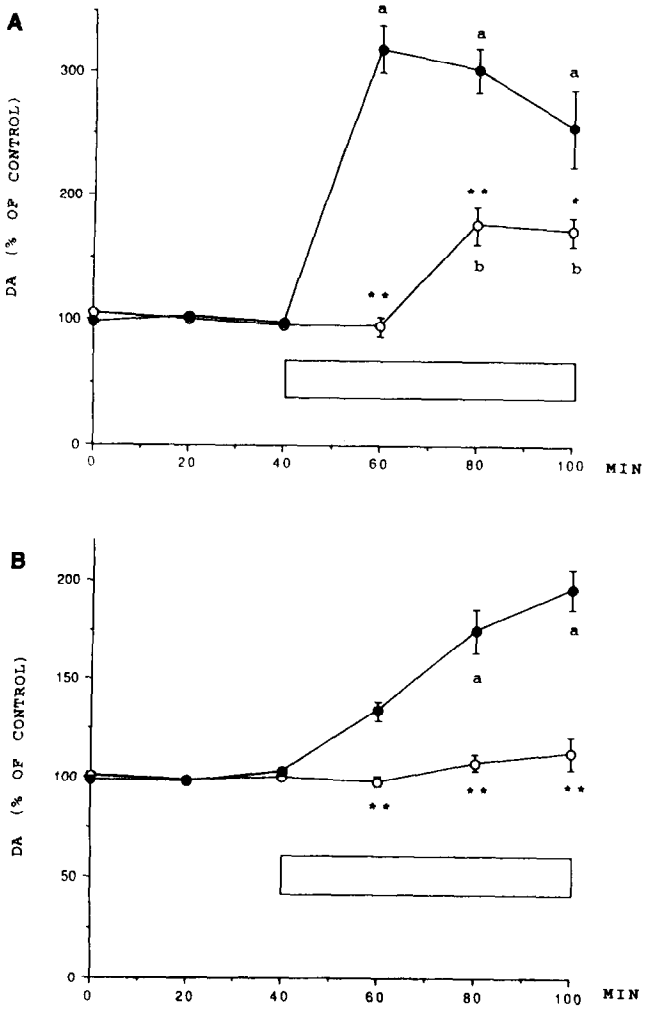

FIG. 2. Effect of NIC on QIN- or SLP-induced increases in DA in the striatum. Quinine (QIN, 1 mM) or sulpiride (SLP, 10^{-5} M) was infused for 1 h in the absence (●, n = 4–5) or presence (○, n = 4) of NIC (1 mM). The mean of three samples with NIC (1 mM) alone was regarded as the new baseline (100%, ○), because DA concentrations had been stabilized during NIC infusion for 1 h. Each point represents the mean ± SEM. NIC significantly blocked QIN-induced increases in DA release (A). NIC also significantly blocked SLP-induced increases in DA release (B). A rectangle indicates drug infusion. Panel A: (a) $p < 0.01$ vs. control (one-way ANOVA and Newman-Keuls test). (b) $p < 0.05$ vs. control (one-way ANOVA and Newman-Keuls test). *$p < 0.05$ vs. QIN 1 mM (two-way ANOVA and Newman-Keuls test). **$p < 0.01$ vs. QIN 1 mM (two-way ANOVA and Newman-Keuls test). Panel B: (a) $p < 0.01$ vs. control (one-way ANOVA and Newman-Keuls test). **$p < 0.01$ vs. SLP (10^{-5} M) (two-way ANOVA and Newman-Keuls test).

Effect of NIC on QIN- or SLP-Induced Changes in Extracellular Levels of DA

In the presence of NIC (10^{-3} M), QIN (10^{-3} M)-induced increases in extracellular DA concentration were significantly blocked by NIC (Fig. 2A). Coadministration of NIC (10^{-3} M) with SLP (10^{-5} M) completely blocked SLP-induced increase in DA release in the striatum (Fig. 2B).

DISCUSSION

There are several reports highlighting the mechanisms by which potassium channels modulate the function of DA autoreceptors in vitro (11,14,15,21). Most of the regions investigated were DA cell body sites studied with the patch clamp technique, because this technique was the only one available for cell body sites as opposed to nerve terminal sites. On the other hand, the in vivo microdialysis technique can be used in both DA cell body sites and dopaminergic nerve terminals (13,22,28,32). Moreover, we can evaluate presynaptic DA autoreceptor function in vivo using this technique (12,26).

This is the first report to indicate that intrastriatal administration of the K_{ATP} channel opener (31) NIC produced dose-dependent decreases in extracellular DA levels in the striatum in vivo. There are some possible mechanisms by which intrastriatal infusion of NIC produced a decrease of extracellular DA level in the striatum. The K_{ATP} channel opener produced a large hyperpolarization of dissociated rat substantia nigra neurons in the rat (10). In addition, the hyperpolarization produced by the D_2 receptor agonist quinpirole is mediated by activation of K_{ATP} channels in substantia nigra neurons in the guinea pig (21). Therefore, one possibility is that NIC produced hyperpolarization and shortening of the duration of action potentials by opening K_{ATP} channels, both of which led to a reduction of Ca^{2+} influx through voltage-dependent Ca^{2+} channels (25). NIC then inhibited exocytotic DA release from nigrostriatal DA-ergic nerve terminals.

Another possibility is that NIC decreased DA release by decreasing Ca^{2+} release from intracellular stores (29). The last possibility is that NIC may directly close voltage-dependent Ca^{2+} channels by hyperpolarization, which then decreases exocytotic DA release in the striatum (31).

Intrastriatal infusion of the D_2 receptor antagonist SLP produced increases in extracellular DA concentrations. This result is consistent with previous in vivo microdialysis studies (12,26,28). SLP increases DA release by inhibiting presynaptic release modulating DA autoreceptors in the striatum (28). The present study indicates that SLP-induced increases in DA release are completely blocked by NIC infusion. This finding suggests that inhibition of presynaptic DA autoreceptors by SLP is blocked by activation of K_{ATP} channels in the striatum. It has also been reported that tolbutamide, which blocks K_{ATP} channels (1), reversed quinpirole (D_2 receptor agonist)-induced

membrane hyperpolarization (21). Together with this report, our findings indicate presynaptic DA autoreceptors in the striatum decrease DA release through activation of K_{ATP} channels in nigrostriatal dopaminergic nerve terminals. These results strongly confirm other in vitro studies suggesting that D_2 receptors are coupled to K_{ATP} channels (10,21).

Diazoxide has been reported to open K_{ATP} channels in pancreatic β cells at concentrations of 10^{-5}–10^{-4} M (34). The dose of NIC used was similar to those used in these previous studies, because only about 10% of the NIC would escape the dialysis membrane (18). Thus, in our study $< 10^{-4}$ M of NIC would reach the striatum through the dialysis membrane to inhibit SLP- or QIN-induced increases in DA levels.

[^{3}H]Glibenclamide, which binds with high selectivity to K_{ATP} channels of central neurons (5), has binding sites that are widely distributed throughout the brain (8,16). For example, K_{ATP} channels are present in the substantia nigra (16), cerebral cortex (3,19), hypothalamus (4), locus coeruleus (7), thalamus (33), and caudate-putamen (33). We have previously reported that local application of QIN, which has recently been regarded as a K_{ATP} channel blocker (6), produced dose-dependent increases in DA release (26). Moreover, the present study demonstrates that local infusion of a K_{ATP} channel opener, NIC, produced dose-dependent decreases in DA release and QIN-induced increases in DA release in the striatum that can be blocked by NIC. These findings strongly suggest that K_{ATP} channels exist in the rat striatum and that inhibition or activation of K_{ATP} channels in this region produce increases or decreases in DA release in vivo, respectively. These results are consistent with both autoradiographic studies (33) and in vitro electrophysiologic studies (1,21,23).

In conclusion, we showed that intrastriatal infusion of a K_{ATP} channel opener, NIC, decreased, whereas similar infusion of a K_{ATP} channel blocker, QIN, increased, DA release in the rat striatum. NIC blocked QIN- or SLP-induced increases in extracellular DA levels in vivo. These results suggest that activation or inhibition of K_{ATP} channels decreases or increases endogenous DA release in the rat striatum in vivo, respectively. Furthermore, DA autoreceptors inhibit DA release through activation of K_{ATP} channels in vivo, because the SLP-induced DA increase was blocked by the activation of K_{ATP} channels.

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